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ORIGINAL REPORT

Relative Abundance of *EGFR* Mutations Predicts Benefit From Gefitinib Treatment for Advanced Non–Small-Cell Lung Cancer

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ABSTRACT

Purpose
Our aim was to determine whether abundance of epidermal growth factor receptor (*EGFR*) mutations in tumors predicts benefit from treatment with EGFR–tyrosine kinase inhibitors (TKIs) for advanced non–small-cell lung cancer (NSCLC).

Patients and Methods
We detected *EGFR* mutations in 100 lung cancer samples using direct DNA sequencing and amplification refractory mutation system (ARMS). Mutation-positive tumors by both methods carried high abundance of *EGFR* mutations. Tumors that were mutation positive by ARMS but mutation negative by direct DNA sequencing harbored low abundance of *EGFR* mutations. Mutation-negative tumors by both methods carried wild-type *EGFR*. All patients received gefitinib treatment. The correlation between *EGFR* mutation abundance and clinical benefit from gefitinib treatment was analyzed.

Results
Of 100 samples, 51 and 18 harbored high and low abundances of *EGFR* mutations, respectively; 31 carried wild-type *EGFR*. Median progression-free survival (PFS) was 11.3 (95% CI, 7.4 to 15.2) and 6.9 months (95% CI, 5.5 to 8.4) in patients with high and low abundances of *EGFR* mutations, respectively ($P = .014$). Median PFS of patients with low abundance of *EGFR* mutations was significantly longer than that of those with wild-type tumors (2.1 months; 95% CI, 1.0 to 3.2; $P = .010$). Objective response rates (ORRs) were 62.7%, 44.4%, and 16.1%, and overall survival (OS) rates were 15.9 (95% CI, 13.4 to 18.3), 10.9 (95% CI, 2.7 to 19.1), and 8.7 months (95% CI, 4.6 to 12.7) for patients with high abundance of *EGFR* mutations, low abundance of *EGFR* mutations, and wild-type *EGFR*, respectively. The difference between patients with high and low abundances of *EGFR* mutations was not significant regarding ORR and OS.

Conclusion
The relative *EGFR* mutation abundance could predict benefit from EGFR-TKI treatment for advanced NSCLC.

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INTRODUCTION

Lung cancer is a leading cause of cancer-related mortality both worldwide and in China.[1,2] More than 70% of patients with lung cancer are diagnosed with advanced non–small-cell lung cancer (NSCLC), and prognosis remains poor. Targeting the epidermal growth factor receptor (EGFR) is a promising strategy for treating NSCLC; the EGFR tyrosine kinase inhibitors (TKIs) gefitinib and erlotinib have been shown to be effective for advanced NSCLC.[3,4] However, the efficacy of TKIs is not consistent for every patient; some patients experience dramatic response to EGFR-TKIs, whereas others show no response. Valid predictive factors of the efficacy of EGFR-TKIs are important for selecting patients who may benefit more from EGFR-TKI treatment. Clinical characteristics such as being a never-smoker, being female, having adenocarcinoma, and being of Asian ethnicity have been predictive factors for the efficacy of EGFR-TKIs, because patients with these characteristics have had better responses than those without them.[5] In 2004, *EGFR*-activating mutations were identified[6,7] and then confirmed to be a better predictor of the efficacy of EGFR-TKI treatment than EGFR protein expression or *EGFR* gene copy number.[8-10] The most common *EGFR*-activating mutations

are in-frame deletions in exon 19 and mutations in exon 21 that result in amino acid substitutions. These mutations are clustered near the ATP cleft of the TK domain, which is targeted by EGFR-TKIs.[11] These mutations might also stabilize the interaction between the receptor and both ATP and its competitive inhibitor EGFR-TKIs, thus enhancing the inhibition induced by EGFR-TKIs.[6] Thus, tumors with *EGFR*-activating mutations demonstrate a better response to EGFR-TKIs than those without them.[10,12,13] These *EGFR*-activating mutations have occurred more frequently in never-smokers, those with adenocarcinomas, and Asian patients, which might explain why these patients have had a better response to EGFR-TKIs.[12,13] However, it remains unclear why some patients with *EGFR* mutations have experienced longer PFS of more than 1 year, whereas some have had PFS of fewer than 6 months.

Previous studies have demonstrated that *EGFR*-activating mutations arise somatically during tumor formation, and only a proportion of cancer cells in an individual patient carry heterozygous activating mutations, whereas other cancer cells carry wild-type *EGFR*.[14,15] Because only cancer cells carrying *EGFR*-activating mutations gain increased affinity for EGFR-TKIs, we hypothesized that the quantification of *EGFR* mutations might predict the extent of benefit from EGFR-TKIs, and patients whose tumors have a high abundance of *EGFR* mutations might benefit more from EGFR-TKIs than those with a low abundance of *EGFR* mutations. The shorter PFS with EGFR-TKIs in some patients with *EGFR* mutations might result from a low abundance of mutations. In the present study, we detected *EGFR* mutations in tumor samples using two methods concurrently: direct DNA sequencing and Scorpion amplification refractory mutation system (ARMS; DxS, Manchester, United Kingdom). The sensitivity of these two methods has been shown to be different.[16] Among commonly used methods for detecting *EGFR* mutations, sequencing showed the lowest sensitivity, whereas ARMS using unimolecular fluorescent probes demonstrated higher mutation-detecting capability.[16-18] Sequencing can detect *EGFR* mutations in samples with a greater than 10% *EGFR* mutation frequency, whereas ARMS can detect mutations in samples with an *EGFR* mutation frequency as low as 1%.[16] Thus, in the present study, we categorized patients into different groups according to the relative abundance of *EGFR* mutations in their tumors, as detected by these two methods, and analyzed the correlation between *EGFR* mutation abundance and clinical benefit from EGFR-TKI treatment.

PATIENTS AND METHODS

Study Design

Tumor samples retrieved from the tumor tissue bank of the Guangdong Lung Cancer Institute (GLCI) had to meet the following criteria: sufficient for two tests and obtained at diagnosis from patients with advanced NSCLC who received gefitinib treatment at any time during the course of their disease. Informed consent was obtained from each patient. DNA extracted from tumor tissues was analyzed for *EGFR* mutations in exons 18 to 21 using both sequencing and ARMS assays. Patients were categorized into three groups according to their *EGFR* mutation status: those with mutation-positive tumors by both methods were in the high abundance of *EGFR* mutations group (group H); those whose tumors were mutation positive by ARMS but negative by sequencing were in the low abundance of *EGFR* mutations group (group L), and those with mutation-negative tumors by both methods were in the wild-type group (group W). Clinical data of all patients were from the electronic medical record database of GLCI. We analyzed the correlation between *EGFR* mutation abundance and clinical benefit from EGFR-TKI treatment.

EGFR *Mutation Analysis*

We performed pathologic assessment. All tumor samples were routinely assessed by sectioning, hematoxylin-eosin staining, and visualization under microscope to ensure tumor content was at least 50%. If the tumor content was observed less than 50%, we trimmed these samples to satisfy the criteria. Mutation analysis of the EGFR-TK domain was performed. Genomic DNA was extracted using DNeasy Blood and Tissue Kit (No. 69504; Qiagen, Valencia, CA), and exons 18 to 21 were amplified with four pairs of primers. Polymerase chain reaction (PCR) products were purified and labeled using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA), followed by sequencing in an ABI 3100 Genetic Analyzer (Applied Biosystems). Both forward and reverse sequencing reactions were performed with respective primers. All sequence variations were confirmed by multiple independent PCR amplifications and repeated sequencing reactions.[6]

The same DNA samples for sequencing were also tested for *EGFR* mutations using ARMS, according to the protocol of the DxS *EGFR* mutation test kit (DxS). A total of 29 mutations in the *EGFR* gene can be detected using this kit. All quantitative PCR (qPCR) reactions were performed using the LightCycler 480 instrument (Roche Applied Science, Indianapolis, IN).

Mutant EGFR Protein Expression by Immunohistochemistry

Rabbit mAbs against EGFR E746-A750del, L858R, and total EGFR protein were from Cell Signaling Technology. For samples with exon 19 deletion detected by ARMS only, mutant-enriched PCR-based sequencing was performed to screen out E746-A750del mutation. Immunohistochemistry (IHC) was performed, and staining intensity was scored from – (complete absence of staining or faint staining intensity in $< 10\%$ cells) to $+++$ (tumor cells had strong staining).

KRAS, BRAF, PI3KCA, ALK, *and* cMET *Alteration Analysis*

For tumors that were *EGFR* mutation positive by ARMS but negative by sequencing, genetic alterations of *KRAS*, *BRAF*, *PI3KCA*, *ALK*, and *cMET* were assessed. The purpose was to exclude their potential effect on the benefit from gefitinib treatment. For *KRAS*, *BRAF*, and *PI3KCA* mutation analyses, genomic DNA was amplified and sequenced as previously described.[19] *cMET* amplification was tested by qPCR as reported.[20,21] The *ALK* fusion status was tested by the methods we have reported.[22]

Study Treatment and Assessments

Patients received gefitinib as second- or third-line treatment in clinical practice or trial (INTEREST [Iressa Non–small-cell lung cancer Trial Evaluating REsponse and Survival against Taxotere] study) after failure of systemic chemotherapy or as first-line therapy in the IPASS (Iressa Pan-Asia Study) trial. Some nontrial patients received gefitinib as first-line therapy, because they refused chemotherapy or could not tolerate chemotherapy as a result of poor performance status or inadequate organ function. Gefitinib was administered orally (250 mg) once daily until disease progression according to RECIST (Response Evaluation Criteria in Solid Tumors) criteria, intolerable toxicity, or patient refusal. Before treatment, each patient underwent physical examination, laboratory tests, electrocardiograms, and chest and upper abdomen computed tomography scans. Brain magnetic resonance imaging or radionuclide bone scans were added when brain or bone metastasis was suspected. The medical history, concomitant medications, and smoking status of each patient were documented. The objective tumor response was assessed every 6 weeks (in clinical trials) or every 8 weeks (in clinical practice). Additional assessment could be performed at any time when symptoms or signs suggested that disease might be progressive. Adverse events were assessed according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0.

Statistical Analyses

The primary end point, progression-free survival (PFS), was defined as the time from commencement of gefitinib treatment to disease progression according to RECIST criteria or death resulting from any cause. Secondary end points included objective response rate (ORR), overall survival (OS), and safety profile. OS was calculated from commencement of gefitinib treatment

to death resulting from any cause.[13] PFS and OS were analyzed using the Kaplan-Meier method and compared between different groups using the log-rank test. Comparison of ORRs in different groups was performed using χ^2 tests. We used multivariate Cox proportional hazards regression model to evaluate independent predictive factors associated with PFS. A two-sided P value of less than .05 was considered statistically significant. To reflect different sensitivities of the two methods (1% by ARMS, approximately 10% to 20% by sequencing), 80 to 214 patient cases were needed by two correlated/paired proportions (using ratios $\alpha = 0.05$, $1\text{-}\beta = 0.90$).

RESULTS

Patient Characteristics

A total of 100 patients were screened and fully met the enrollment criteria. The characteristics of the patients were as follows: 51 women; 49 men; median age of 55 years (range, 23 to 84 years); 77 never-smokers and 23 former/current smokers; 93 adenocarcinomas, six squamous cell carcinomas, and one adenosquamous carcinoma; and 11 instances of TNM stage IIIB and 89 of TNM stage IV. A total of 26 patients received gefitinib treatment as first-line, 42 as second-line, and 32 as third-line therapy. We detected *EGFR* mutations in 51 samples (51%) by both sequencing and ARMS (group H). Of 49 samples that were *EGFR* mutation negative by sequencing, 18 were positive (group L) and 31 were negative (group W) by ARMS. The characteristics of patients in the three groups are listed in Table 1. Sex, smoking status, and line of TKI therapy were well balanced among these groups. Six of seven patients with nonadenocarcinomas were in group W, and one patient with adenosquamous carcinoma was in group L. All 51 patients in group H had adenocarcinomas. The distribution of pathologic type among the three groups was significantly different ($P = .002$), but that between groups H and L demonstrated no significant difference ($P = .261$).

Table 1. Patient Demographics and Clinical Characteristics

Characteristic	Group H* No.	Group H* %	Group L† No.	Group L† %	Group W‡ No.	Group W‡ %	P§
Sex							.421
Male	22	43.1	9	50.0	18	58.1	
Female	29	56.9	9	50.0	13	41.9	
Age, years							
Median	54.5		51.5		56		
Range	27-84		37-69		23-75		
Smoking status							.151
Never-smoker	42	82.4	15	83.3	20	64.5	
Smoker	9	17.6	3	16.7	11	35.5	
Pathology							.002‖
Nonadenocarcinoma	0	0	1	5.6	6	19.4	
Adenocarcinoma	51	100	17	94.4	25	80.6	
Line of TKI therapy							.271
First	9	17.6	5	27.8	12	38.7	
Second	25	49.1	6	33.3	11	35.5	
Third	17	33.3	7	38.9	8	25.8	
Proportion of trial patients							
Trial	4	7.8	4	22.2	3	9.7	.322
Nontrial	47	92.2	14	77.8	28	90.3	

Abbreviations: ARMS, amplification of refractory mutation system; TKI, tyrosine kinase inhibitor.
*Mutation positive by both DNA sequencing and ARMS.
†Mutation negative by DNA sequencing, positive by ARMS.
‡Mutation negative by both DNA sequencing and ARMS.
§Comparison among three groups.
‖After partitioning of χ^2 analysis, difference in pathologic distribution was found between groups H and W ($P = .002$); differences between groups H and L and groups L and W were not significant ($P = .261$ and .238, respectively).

EGFR *Mutation Status*

In 100 tumor samples, we found 29 deletion mutations in exon 19, 16 point mutations of L858R in exon 21, one L861Q mutation in exon 21, one L858R and T790M double mutation, one deletion and T790M double mutations, and three L858R and deletion double mutations by sequencing. These mutations were all confirmed by ARMS. Additionally, we found eight deletion mutations in exon 19, seven L858R mutations in exon 21, one L861Q mutation in exon 21, and two deletion and T790M double mutations by ARMS in 49 mutation-negative samples by sequencing. The other 31 samples were mutation negative by both methods. An 82% concordance was observed between sequencing and ARMS. In total, 18 patients were detected with discordant mutation status by the two methods. These 18 *EGFR* mutation–negative samples were detected by sequencing, including 17 adenocarcinomas and one adenosquamous carcinoma, which were found to be mutation positive by ARMS. No significant difference was observed in the distribution of mutation type between groups H and L ($P = .491$). Additionally, incidence of T790M mutation was not different between groups H and L ($P = .277$).

When we pooled groups H and L into the analysis, *EGFR* mutations were more common in samples with adenocarcinoma than in those with nonadenocarcinoma ($P = .003$) and more common in never-smokers than in smokers ($P = .047$). Mutation rates by sex demonstrated no significant difference ($P = .224$).

Mutant EGFR Protein Expression by IHC

Of the 100 patient cases, 12 specimens were large enough for IHC after use of sequencing and ARMS assays. Seven patients were in group

Table 2. IHC Results of 12 Samples

Group*	Mutations by Sequencing	Mutations by ARMS†	IHC Results‡ E746-750 del	IHC Results‡ L858R
L	Negative	Exon 19 deletion	+	−
L	Negative	Exon 19 deletion	+	−
L	Negative	Exon 19 deletion	−	−
L	Negative	L858R	−	++
L	Negative	L858R	−	−
L	Negative	L858R	−	++
L	Negative	L858R	−	+
H	E746-A750del	Exon 19 deletion	++	−
H	E746-A750del	Exon 19 deletion	−	−
H	E746-A750del	Exon 19 deletion	+++	−
H	L858R	L858R	−	+++
H	L858R	L858R	−	+++

Abbreviations: ARMS, amplification of refractory mutation system; IHC, immunohistochemistry.
*Group L, mutation negative by DNA sequencing, positive by ARMS; group H, mutation positive by both DNA sequencing and ARMS.
†Exon 19 deletion was confirmed as E746-A750del by mutant-enriched polymerase chain reaction–based sequencing.
‡−, complete absence of staining or faint staining intensity in < 10%; +, > 10% tumor cells had faint staining; ++, tumor cells had moderate staining; +++, tumor cells had strong staining.

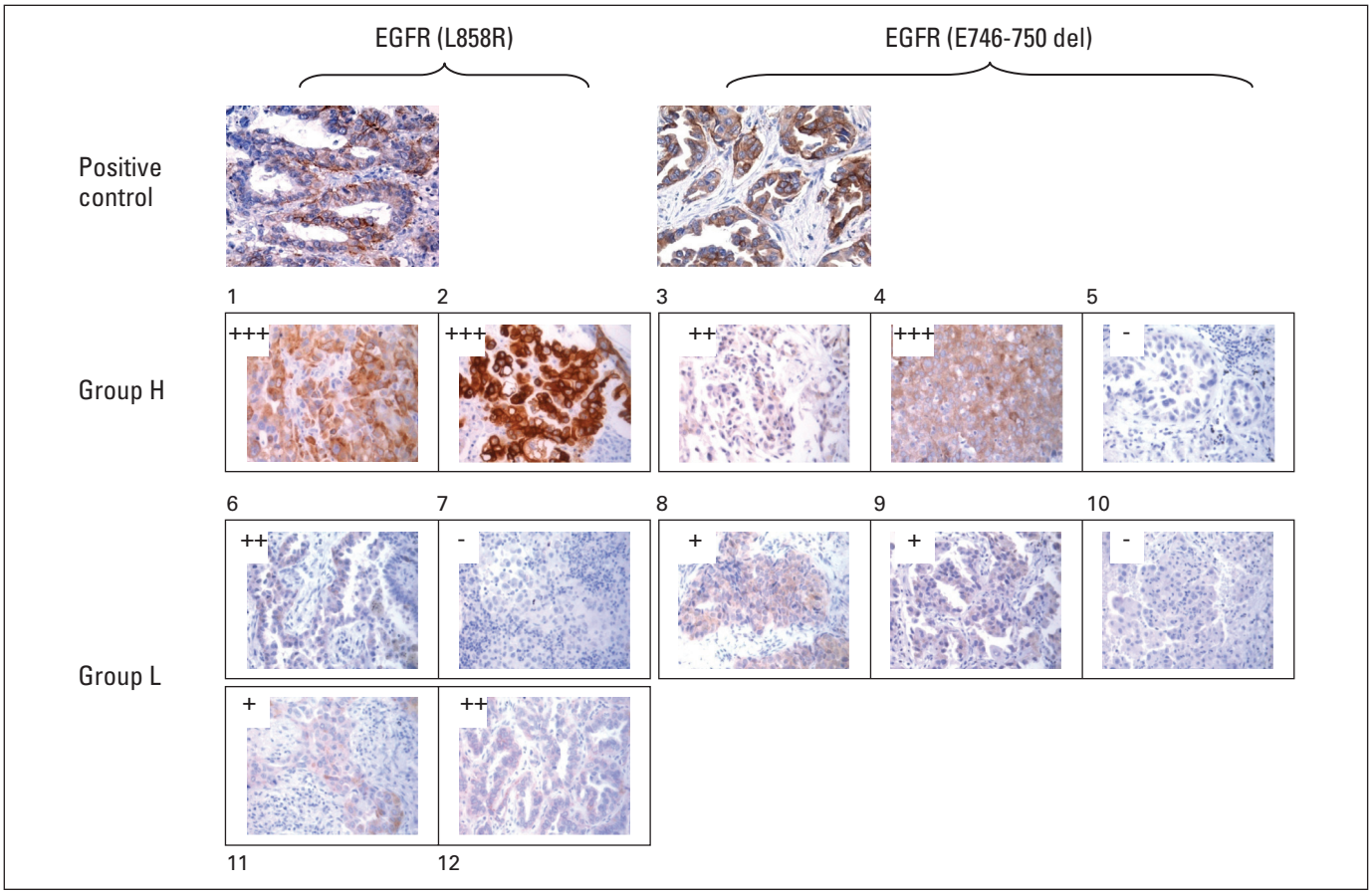

Fig 1. Immunohistochemistry (IHC) results of 12 samples with epidermal growth factor receptor (*EGFR*) L858R or E746-750 del mutations. Three of five patients in group H showed strong intensity (+++) of mutant EGFR protein expression, whereas none of seven patients in group L showed such strong expression of mutant EGFR proteins (−, complete absence of staining or faint staining intensity in < 10%; +, > 10% tumor cells had faint staining; ++, tumor cells had moderate staining; +++, tumor cells had strong staining). Two lung cancer samples detected previously by both sequencing and IHC in our laboratory were used as positive controls. All photographs taken at ×200 magnification.

L, and five were in group H. Three patients in group H showed strong intensity, whereas none of seven patients in group L showed such strong expression (Table 2; Fig 1).

KRAS, BRAF, PIK3CA, cMET, *and* ALK *Status in EGFR Sequencing-Negative, ARMS-Positive Tumors*

In 10 EGFR sequencing-negative, ARMS-positive tumors with adequate DNA samples, we found no abnormality in the *KRAS*, *BRAF*, *PIK3CA*, and *cMET* genes. *ALK* fusion was also not observed.

Efficacy and Toxicities

All 100 patients commenced gefitinib treatment between April 2006 and September 2009 (Table 3). The last follow-up date was April 30, 2010, and median follow-up duration was 22.5 months (range, 2.5 to 48.0 months). Ninety-five patients exhibited disease progression. The difference in median PFS among the three groups was significant ($P < .001$; Fig 2). The median PFS of patients in group H (11.3 months; 95% CI, 7.4 to 15.2 months) was significantly longer than that in group L (6.9 months; 95% CI, 5.5 to 8.4 months; $P = .014$). Additionally, the median PFS of patients in group L was significantly longer than that in group W (2.1 months; 95% CI, 1.0 to 3.2 months; $P = .010$). The study met its primary end point of demonstrating that patients with a high abundance of *EGFR* mutations could gain more benefit from gefitinib treatment than those with low abundance of EGFR mutations regarding PFS. In the multivariate Cox proportional hazards regression model, groupings with different mutation abundances, sex, smoking status, line of TKI therapy, and pathology were used as covariates. Results showed that the groupings and smoking status were significantly associated with PFS (hazard ratio [HR] for group W *v* L, 2.977; 95% CI, 1.543 to 5.747; $P = .001$; HR for group H *v* L, 0.513; 95% CI, 0.286 to 0.923; $P = .026$; HR for never-smokers *v* smokers, 2.432; 95% CI, 1.292 to 4.577; $P = .006$).

The ORRs in groups H, L, and W were 62.7%, 44.4%, and 16.1%, respectively. The difference in ORR between groups H and W was significant ($P < .001$), whereas those between groups H and L and between groups L and W were not ($P = .176$ and .067, respectively). At the last follow-up date, 82 patients had died. The median OS rates of patients in groups H, L, and W were 15.9 (95% CI, 13.4 to 18.3), 10.9 (95% CI, 2.7 to 19.1), and 8.7 months (95% CI, 4.6 to 12.7), respectively. Similar to the ORR, the difference in the median OS between groups H and W was significant ($P = .005$), whereas those between groups H and L and between groups L and W were not ($P = .062$ and .472, respectively; Fig 3). These results show that patients with a high

Table 3. Efficacy of Gefitinib Treatment

Efficacy	Group H* No.	Group H* %	Group L† No.	Group L† %	Group W‡ No.	Group W‡ %	P§	P‖
PFS, months							< .001	.014
Median	11.3		6.9		2.1			
95% CI	7.4 to 15.2		5.5 to 8.4		1.0 to 3.2			
OS, months							.011	.062
Median	15.9		10.9		8.7			
95% CI	13.4 to 18.3		2.7 to 19.1		4.6 to 12.7			
Tumor response							< .001	.176
CR	2	3.9	0	0	0	0		
PR	30	58.9	8	44.4	5	16.1		
SD	15	29.4	5	27.8	10	32.3		
PD	4	7.8	5	27.8	16	51.6		

Abbreviations: ARMS, amplification of refractory mutation system; CR, complete response; OS, overall survival; PD, progressive disease; PFS, progression-free survival; PR, partial response; SD, stable disease.
*Mutation positive by both DNA sequencing and ARMS.
†Mutation negative by DNA sequencing, positive by ARMS.
‡Mutation negative by both DNA sequencing and ARMS.
§Comparison among three groups.
‖Comparison between groups H and L with partitioning of χ^2.

abundance of *EGFR* mutations in tumors demonstrated a better response to gefitinib and longer OS than both patients with wild-type *EGFR* tumors and those with a low abundance of EGFR mutations in tumors.

Rash and diarrhea were the most common toxicities (75 of 100 and 51 of 100 patients, respectively), and their incidence showed no significant difference among the three groups. Most of the toxicities were grade 1 or 2. Nine patients exhibited grade 3 rash, and no dose reduction or discontinuation occurred because of intolerable toxicities. No interstitial lung disease was observed.

DISCUSSION

To our knowledge, the present study represents the first to demonstrate that the abundance of *EGFR* mutations could predict the extent

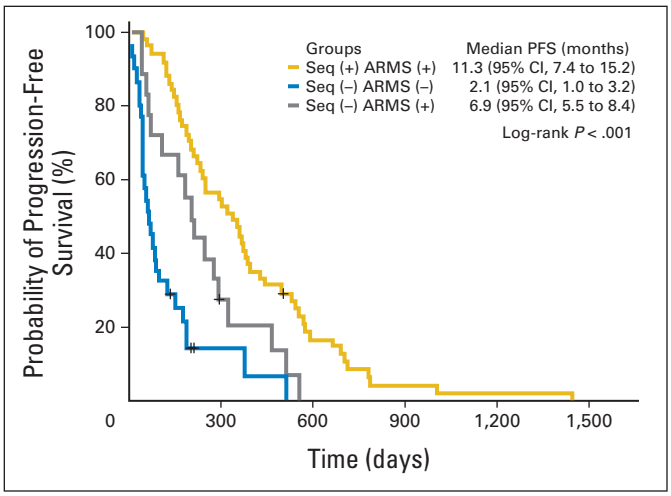

Fig 2. Progression-free survival (PFS) of patients in the three groups. ARMS, amplification refractory mutation system; Seq, DNA sequencing.

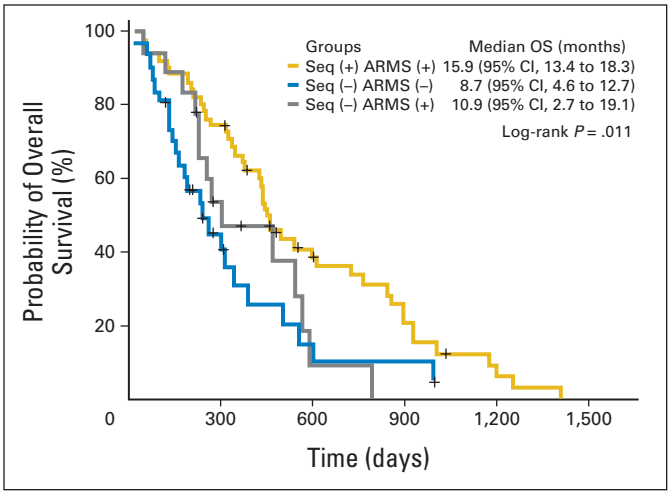

Fig 3. Overall survival (OS) of patients in the three groups. ARMS, amplification refractory mutation system; Seq, DNA sequencing.

of benefit from EGFR-TKI treatment for advanced NSCLC. Thus, a completely new concept is revealed, because previous studies have only focused on whether the mutation is positive. Our results show that patients with a high abundance of *EGFR* mutations may benefit more than those with a low abundance of *EGFR* mutations according to the statistically different PFS between the groups ($P = .014$), although the 95% CIs had some overlap. Notably, the shorter PFS of patients with a low abundance of *EGFR* mutations was not caused by other tumor growth–driving alterations in kinases such as *KRAS*, *BRAF*, *PI3KCA*, *ALK*, and *cMET*. These results might also partly explain why the duration of response of some patients who were *EGFR* mutation positive was not as long as expected when no resistance-related abnormality was found. By precisely quantifying the abundance of *EGFR* mutations, we can select patients with a high abundance of *EGFR* mutations for EGFR-TKI treatment, because they would benefit the most. More importantly, closer attention should be paid to those patients with a low abundance of *EGFR* mutations when treatment strategies are decided; combined modality strategies, instead of EGFR-TKIs alone, might be necessary for these patients.

Although direct sequencing is regarded as a classic method for *EGFR* testing,[6,7] ARMS demonstrated higher sensitivity at 0.1% to 1%.[16,23,24] In this study, we detected *EGFR* mutations in the same sample set using two methods with low and high sensitivity concurrently. For each patient, both *EGFR* mutation testing methods were based on the same DNA sample extracted from the tumor, avoiding inconsistencies in mutation status testing that could potentially result from intratumor heterogeneity of genetic abnormalities. Thus, the discordant mutation status by sequencing and ARMS could be substantially explained by the different sensitivities for detecting a low abundance of mutations. IHC results were also consistent to the categorization of *EGFR* mutation abundance by ARMS and sequencing, although the implication of heterogeneity of staining could not be clearly addressed because of the unavailability of all specimens in group L. Theoretically, false positivity by ARMS might still exist, especially if *EGFR* mutation frequency were extremely low. To exclude potential false positivity and get actual intratumor abundance of *EGFR* mutations, we need more advanced biotechniques, such as

microfluidics digital PCR.[25] In addition, one other potential contribution to low abundance is amplification of wild-type *EGFR* allele, which might be clarified by fluorescent in situ hybridization or specific technology for wild-type alleles.

An advantage of the present study is that it was performed based on two databases: tumor tissue bank and the electronic medical record database at GLCI. The tumor tissue bank was established in 1999, and almost all patients with lung cancer who have undergone surgery or invasive diagnostic procedures have had their tumor tissues stored at the tumor bank. Informed consent was obtained from each patient. The electronic medical record database was set up in 2004, and the clinical data of each patient were well documented. These two databases increased the reliability of the results. A limitation of the present study is that clinical response assessment was performed every 6 weeks for trial patients but every 8 weeks for nontrial patients. Nonetheless, the proportion of trial patients in the three groups was not significantly different. One other issue is the limited sample size of 100 patient cases. However, results demonstrated a trend of high abundance of *EGFR* mutations, favoring more benefits from TKI. This would be helpful in the future design of a prospective study.

In summary, our study suggests that the relative abundance of *EGFR* mutations could predict the extent of benefit from EGFR-TKI treatment. Precise quantification of *EGFR* mutation abundance can not only can promote a better selection of patients for EGFR-TKI treatment but also help develop better treatment strategies for patients with a low abundance of *EGFR* mutations. A prospective study using accurate mutation quantification techniques is warranted in the future.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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